FINAL REPORT

Aquatic Surveillance
1965 Malathion Test Project

Salmon National Forest

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		Page
I.	Introduction	1
II.	Drift Samples	3
III.	Drift Aquaria Tests	12
IV.	Bottom Samples	15
V.	Live-Box Tests	20
VI.	Cholinesterase Tests	22
VII.	Conclusions	25

I. INTRODUCTION

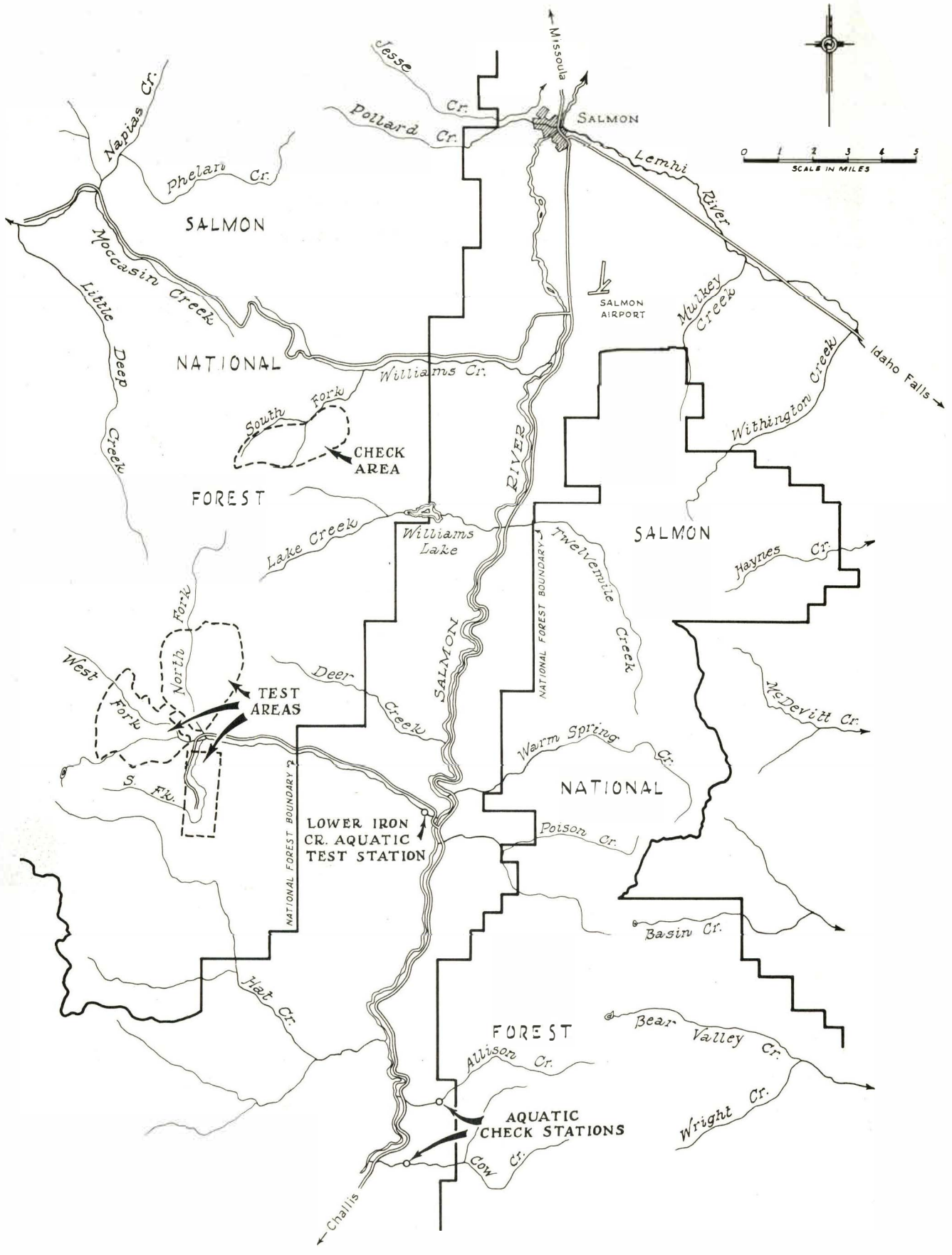
In 1965, the U. S. Forest Service undertook an aerial spray project to test the effects of low volume applications of technical grade malathion on spruce budworm. Iron Creek, a small tributary to the Salmon River, was selected as the testing site. The major objectives of this program were to test the effects of two rates of application of technical grade malathion, as applied by two different types of aircraft, on spruce budworm. Secondly, to evaluate the toxic effects of malathion spray on both aquatic and terrestrial organisms, other than the target insect.

Iron Creek is formed by three major forks all converging within 300 yards of each other. These forks are the West, South and North Forks (see 1965 Malathion Test Project Map). At the time of spraying the West, South, and North Forks were flowing at the approximate rates of 35, 15, and 40 c.f.s. respectively. From the confluence of the North Fork with the West Fork, the main channel of Iron Creek drops rapidly for a distance of eight miles to the main Salmon River.

On July 9, 10, and 11 approximately 2900 acres of the West Fork drainage were sprayed with a 13 fluid ounce application of malathion. Spray was applied by one Steerman and one Callair aircraft. On July 12, approximately 3950 acres within the North Fork drainage were sprayed with 9 ounces of malathion with a TBM aircraft. On July 13, some 1300 acres were sprayed in the South Fork drainage by a TBM aircraft applying 13 ounces per acre. The malathion applied was technical grade material without solvent or carrier.

In general, flight patterns while spraying were parallel to the main stream channels. Spray pilots were instructed to place a spray swath on each side of the test streams in such a manner that the lower edge of the swaths coincided with the edge of the stream channel. No effort was made to avoid the stream with non-spray protection areas. All three forks were sprayed in the above manner.

1965 MALATHION TEST PROJECT



II. DRIFT SAMPLES

Methods

Series of drift samples, consisting of one five-minute sample at each station, were taken throughout the spray area prior to, during and after spraying to determine the immediate and toxic effects of malathion spray on aquatic insects. These samples were collected at the lower and middle portions of each spray area (see spray area map on next page). Additional samples were taken at the lower end of Iron Creek proper, (page 2). Drift sample collection sites were located prior to spraying and marked with wooden stakes. When possible, sites were located near the center of the main current in water 12 to 13 inches deep. These depths allowed the 12-square-inch openings on Surber nets to be fished from the bottom of the stream bed to the surface of the water.

All drift samples were collected with Surber nets with wings folded, figures 3. Each drift sample was collected over a five-minute period each hour. The drift mass from each daytime sample was examined and all aquatic insects were removed and placed in glass bottles. Drift masses collected at night were placed intact in sample bottles and sorted in the laboratory. Samples were preserved in alcohol.

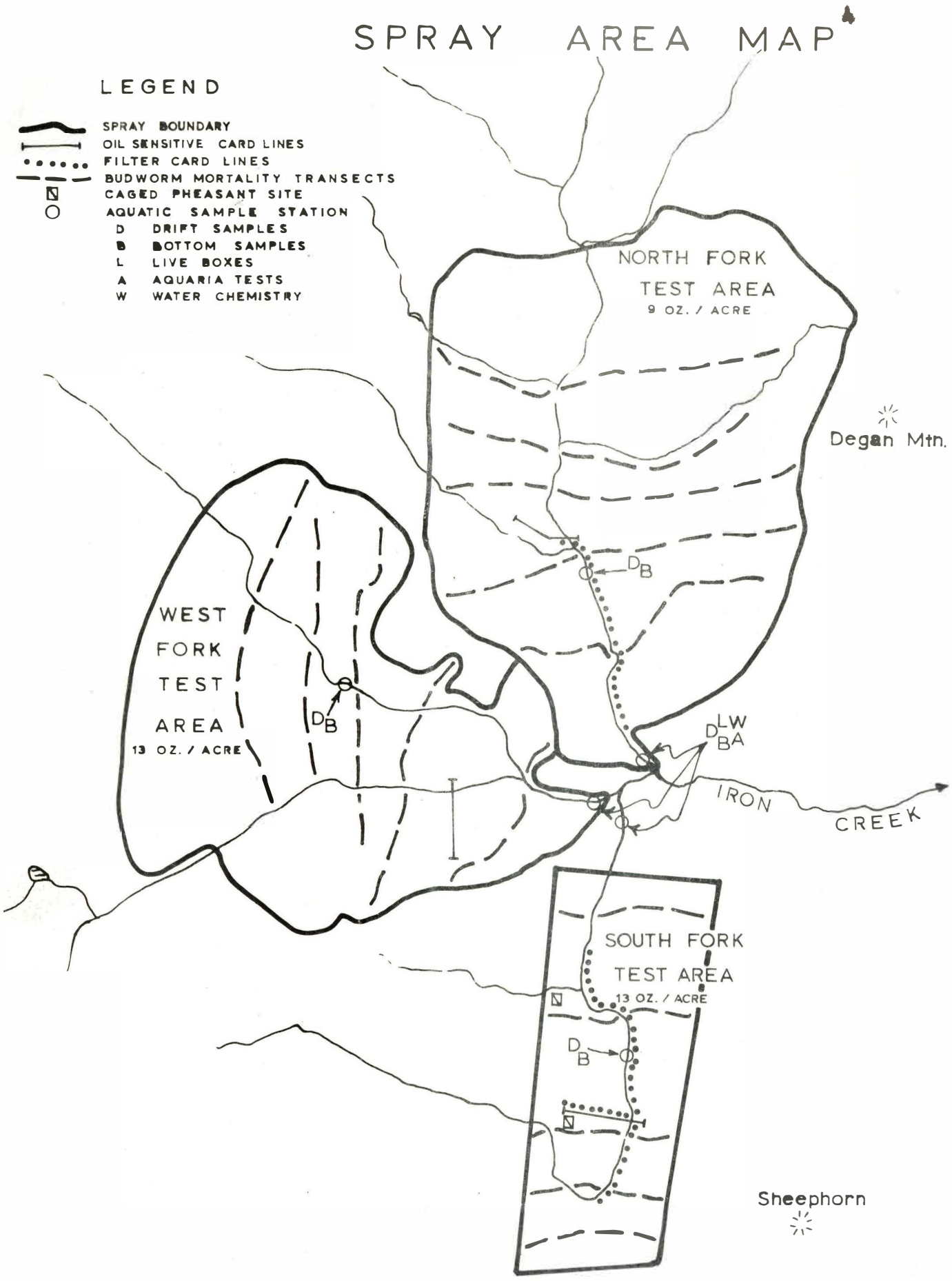
At the conclusion of the project, forest entomologists and fisheries biologists examined each sample individually. Aquatic insects were classified by orders. In addition, both the number and volume of each order of insects in individual samples were recorded. Volumes were measured to the nearest one tenth of a cubic centimeter.

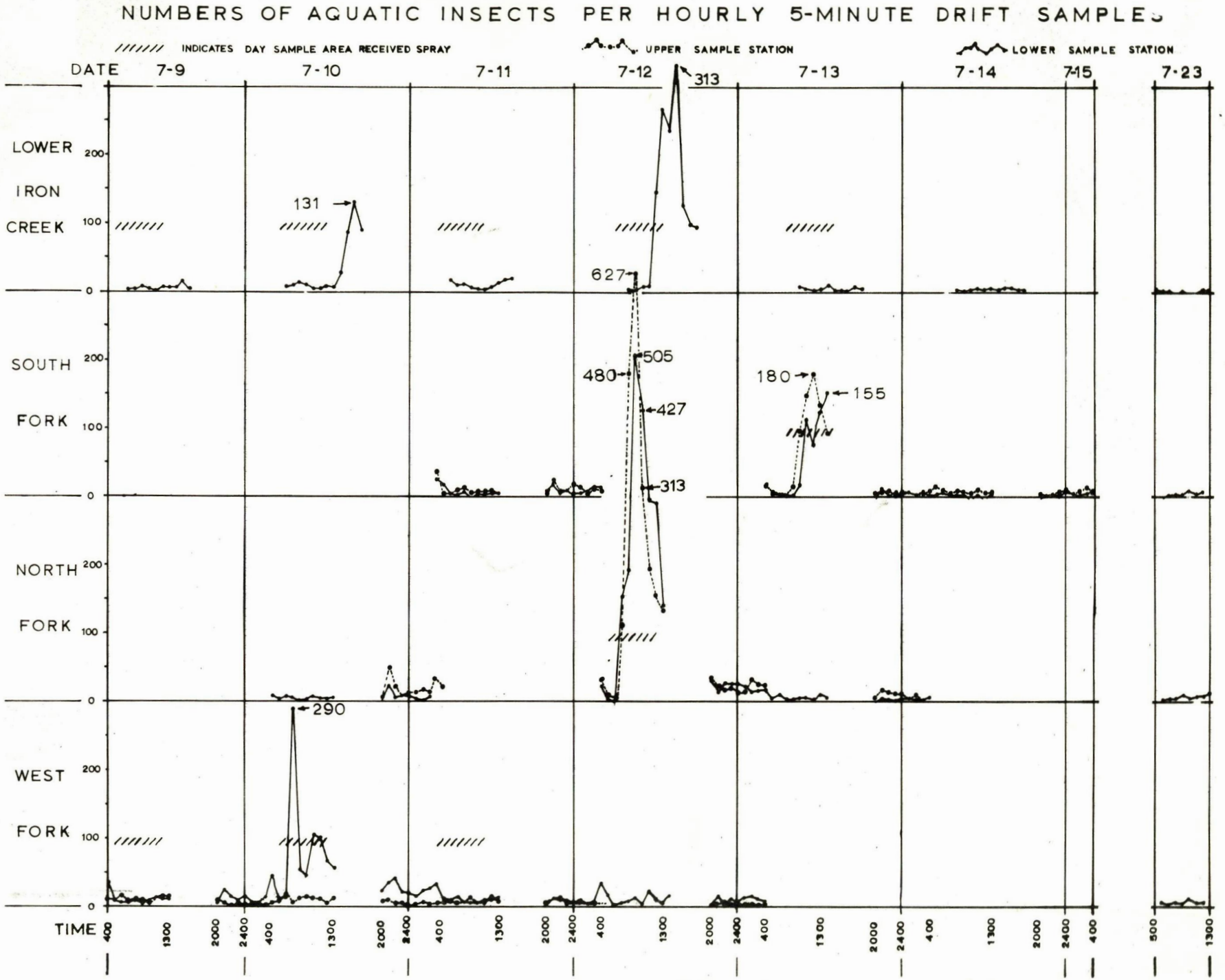
Results

The results of aquatic insect drift sampling are shown in the tables on pages 7-8. From these data it is apparent that substantial numbers of insects were affected by malathion spray. It is also apparent that increases of drifting insects on spray days were primarily composed of the smaller forms of Ephemeroptera and Plecoptera as evidenced by a lack of correlation between large numbers and small volumes. This fact is more apparent when numbers and volume of Ephemeroptera and Plecoptera from bottom samples are compared. The latter data may be seen in a following section of this report.

The tables both reveal a general reduction in average number of drifting insects when comparing pre- and post-spray sampling periods at all stations. A reduction in numbers of drifting insects of approximately 46 percent, occurred between the pre- and 24-hour post-spray samples collected during the hours of darkness. Similarly, a 33 percent recuction occurred in samples collected during the daytime. The average number of insects in post-spray daytime samples taken on July 23 shows an approximate 50 percent reduction over the pre-spray average.

SPRAY AREA MAP




NUMBERS OF AQUATIC INSECTS PER HOURLY 5-MINUTE DRIFT SAMPLES
INDICATES DAY SAMPLE AREA RECEIVED SPRAY
UPPER SAMPLE STATION
LOWER SAMPLE STATION
DATE
7-9
7-10
7-11
7-12
7-13
7-14
7-15
7-23
LOWER IRON CREEK
SOUTH FORK
NORTH FORK
WEST FORK
0
100
200
131
313
627
505
480
427
180
155
290
TIME
400
1300
2000
2400

The chart on page 9 shows comparisons in numbers of drifting insects for pre- and post-spray periods by hours. The upper West Fork station was the only station which did not show an increased drift on at least one spray day. Conversely, both North Fork stations show the largest increases in drifting insects. A prolonged period of increased insect drift occurs when comparing malathion and DDT drift patterns. In 1964, most streams affected by DDT showed that increases in drifting organisms usually lasted for a period of three to five hours. Malathion data indicates that increases in drifting organisms will continue longer than seven hours. It may be that this prolonged drift pattern results from flushing time (period of time necessary for the insecticide to pass downstream) or, possibly due to a slower toxicity reaction of the insecticide with the insect. Regardless of the cause, future surveillance programs with malathion should be planned to fully evaluate the complete drifting pattern.

Pre-spray data shown in chart on page 9 shows the normal trend of increased insect drift during the hours of darkness as compared to daylight hours. Drift increases appear to be bi-modal. The first increase occurred shortly after dusk, the second occuring shortly before dawn.

The chart shows the effects of malathion spray on the Lower Iron Creek station, a downstream station located outside the spray area. This station was subjected to repeated dosages of insecticide over a five day period. Increased insect drift occurred on both July 10 and 12. Corresponding increases occurred at upstream stations on the same dates. However, no increase occurred at this station on July 13, when the upstream South Fork stations both showed substantial increases. This may in part be due to a somewhat reduced population of insects as shown by post-spray bottom samples data, particularly the smaller and apparently more susceptible forms of Ephemeroptera and Plecoptera. The table on page shows that a 68 percent reduction in numbers of insects occurred at this station between pre- and 24-hour post-spray drift samples. A 93 percent reduction took place between pre- and 10-day post-spray samples.

The percentage increases of drifting insects on spray days over the pre-spray levels are shown in the following table.

Pre-Spray Mean	Station	Date	Spray Day Mean: 3 Highest Consecutive Samples	Spray Day Mean: All Samples That Day	Percentage Increase to the Closest 100% Increase: 3 highest Consecutive Samples	Percentage Increase to the Closest 100% Increase: All Samples That Day
11.7	Lower Iron	7/9	9.7	6.1	0	0
		7/10	102.7	33.3	800	200
		7/11	16.3	11.2	0	0
		7/12	278.7	129.6	2300	1000
		7/13	5.7	4.7	0	0

Pre-Spray Mean	Station	Date	Spray Day Mean: 3 Highest Consecutive Samples	Spray Day Mean: All Samples That Day	Percentage Increase to the Closest 100% Increase: 3 Highest Consecutive Samples	Percentage Increase to the Closest 100% Increase: All Samples That Day
12.3	Upper W. Fork	7/9	14.7	11.9	0	0
		7/10	13.0	11.0	0	0
		7/11	10.3	8.9	0	0
10.5	Lower W. Fork	7/9	17.6	11.9	100	0
		7/10	130.7	83.3	1100	700
		7/11	11.7	10.3	0	0
(5.1)*	Upper N. Fork	7/12	473.3	205.9	(9200)	(4000)
5.1	Lower N. Fork	7/12	408.7	201.5	(7900)	(3900)
9.1	Upper S. Fork	7/13	154.3	70.8	1600	700
6.7	Lower S. Fork	7/13	118.0	55.6	1700	700

*No pre-spray samples taken at this station. Average for lower North Fork Station was used.

The three highest consecutive 5-minute samples were used as a basis of determining percentage increase in order to compare the data with last years project. This method was used on the 1964 project as a basis of determining when to move back from the streams.

In order to get a truer picture of increases due to malathion spraying an average of all samples each spray day was also used. The percentage increases for both methods were expressed to the closest 100 percent. Increases of less than 50 percent or decreases were expressed as 0. Zeros appear 50 percent of the time.

III. DRIFT AQUARIA TESTS

Methods

Typical of most spray applications with a chlorinated hydrocarbon or organic phosphorus type insecticide, an above normal number of aquatic insects begin to drift downstream shortly after exposure. In previous spray project reports, these drifting insects were usually described as being moribund. Normally, little or no recover was expected, particularly when DDT or malathion insecticides were used. Therefore, the purpose of these tests was simply to determine if some insect recovery could be expected with low volume applications of malathion. Secondly, if recovery did occur, to approximate the magnitude of recovery.

In order to test the aquatic insect recovery a total of four test stations were selected. These stations correspond with the lower live-box and drift stations on each spray area, as well as the station located on the lower end of main Iron Creek.

Aquaria were constructed from rectangular plastic containers with volumes of approximately two gallons each. Openings about five inches square, were made on three sides of each aquarium and were covered with plastic screening. The plastic screen contained approximately 400 openings per square inch.

Two aquaria were then placed inside of a fine mesh screen cage.

Battery of live-boxes and drift recovery aquaria cage located at the Lower West Fork test station.

Each aquarium was weighted with several fist size rocks for anchoring as well as providing cover for insects placed inside. The top of each aquarium was covered with a fine mesh screen to prevent hatching insects from flying out of the aquaria. The aquaria cages were anchored in the stream and adjusted so that two inches of each aquarium protruded above the surface of the stream. The openings on three sides of each aquarium allowed continuous circulation of water and helped in flushing out fine sediment.

Test insects for each aquaria were obtained by fishing Surber samplers for two minute periods. Normally each aquarium was filled with ten two-minute drift samples. Daytime samples were usually collected immediately after the first five-minute drift sample showed a definite increase in the numbers of drifting insects. Night time samples were collected between 2000 and 2200 hours during the periods of increased drift. No attempt was made to determine the number of dead or live insects at the time samples were collected.

Aquaria were emptied 96 hours after last exposure to the insecticide. All insects were sorted as alive or dead and then preserved in alcohol. Insects were then sorted and counted by orders.

Results

The combined results of all aquaria tests are shown in the following table.

	L.S. Fork	L.W. Fork	L.N. Fork	L.N. Fork	L.S. Fork	L.S. Fork	L. Iron	L. Iron
Prespray Sample	x							
Spray Day Sample		x	x	x	x	x	x	x
Day Samples	x	x	x		x		x	x
Night Samples				x		x		
Ephemeroptera - Live	5	7						
Dead	1	6						
Plecoptera - Live	0	2						
Dead	0	184						
Trichoptera - Live	2	1						
Dead	0	2						
Diptera - Live	6	3						
Dead	0	0						
Coleoptera - Live	0	0						
Dead	1	0						
All orders No. Live	13	13						
Percent Live	86.7	6.3						
No. Dead	2	192						
Percent Dead	13.3	93.7						
Total Live and Dead	15							

The results of the pre-spray test indicated that a small percentage of insects which are normally found drifting downstream will die for one reason or another. However, the sample size in this case was so small that little reliance can be placed on the live-dead ratio.

The investigators recognized several inherent weaknesses in the design of this experiment. However, as mentioned previously, this experiment was primarily designed to determine if any recovery could be expected. Therefore, the resultant recovery rates are at best, rough estimates and certainly not exact ones.

The results of the daytime samples on the lower South and West Fork stations are probably indicative of what can be expected from normal spray operations using a 13 ounce application of malathion. Although these two tests vary in several ways, the fact that both test areas were sprayed with a 13 ounce application with no effort to protect the stream would appear to make them typical of normal operations. In contrast, the stream in North Fork, which was sprayed with only 9 ounces of malathion, apparently received a heavier dosage of insecticide as the result of drifting. The latter observation appears to be substantiated by aquatic insect drift data collected on this stream.

The results of the Lower Iron Creek station shows the highest percentage recovery for daytime samples. These results appear contradictory since this same station also showed the greatest reduction in number of insects as will be shown later in bottom sample results. However, there is one important difference between these samples and those collected at upstream stations. Daytime aquaria samples at the three upstream stations were collected after significant increases in numbers of drifting insects occurred. Those samples collected at the Lower Iron Creek station were taken before any significant increase occurred. This fact is reflected by the small number of insects placed in each aquarium. Therefore, insects placed in the Lower Iron Creek aquaria were normal, meaning they had not been affected by the spray at the time of collections. These facts may, in part, account for differences in the percentages of recovery.

The results of nocturnal samples show a somewhat higher recovery average than do daytime samples. The 50 percent recovery in the South Fork is probably indicative of normal operations. Again, the lower recovery percentage in the North Fork is probably due to the higher concentration of spray from the apparent increased drift over the stream.

One source of error and difficulty in this experiment involved the counting of exoskeletons. Through normal development, some exoskeletons are found in most drift samples. However, there appeared to be an excessive number of exoskeletons in the aquaria at the end of the testing period. This indicates that some insects which died in the acquaria were decomposed to the extent that only the exoskeletons were left. All exoskeletons were recorded as "dead" insects. Since it was impossible to determine which skeletons existed as such before collection, some error resulted in the computations of live-dead ratios.

VI. BOTTOM SAMPLES

Methods

Bottom samples were collected from both test and control streams in order to evaluate the impact of malathion spray on aquatic insect populations. Sampling areas were located in both the lower and middle sections of each test stream. Additional stations were established on lower Iron, Cow, and Allison Creeks, the latter two streams serving as controls (see map on page 2).

Sampling areas were selected, when possible, for uniformity of bottom materials, depth, and flow velocity. Actual sampling locations within the stream channel were pre-determined using a method of random numbers for selecting samples. Pre- and post--sampling locations were located by wooden stakes driven into the stream bottom. Each stake was coded to identify pre- and post-samples.

Sampling apparatus consisted of a circular frame covered with a fine mesh copper screen. The sampler yielded a two square foot sample. When used, the sampler was placed firmly in the gravel and the gravel then stirred with wooden paddles. Larger stones were removed before stirring began, and each examined individually for clinging insects.

A total of five two-square-foot samples were collected during the pre- and two post-spray periods from each of two stations on each test stream. Ten two-square-foot samples were obtained during each sampling period from Lower Iron, Cow and Allison Creek stations.

Insects collected from bottom samples were placed in glass bottles and preserved with alcohol. Each bottle was numbered for identification. Thus, insects in each two square foot sample were identified by orders, counted, and measured for volume. Volumes of each order were measured to the nearest tenth of a cubic centimeter by liquid displacment. Volumes less than one tenth of a cubic centimeter were recorded as a trace.

Results

The following two tables show numerical counts and volumes of aquatic insects collected from bottom samples at all stations. These data indicate a general increase in numbers and volume of insects from pre- to post-spray samples. One exception is the Upper West Fork station, which showed an approximate 34 percent reduction in numbers and reduction in volume of approximate 89 percent at the same station during the same sampling period. Similarly, the Lower West Fork station also showed a reduction in volume amounting to about 12 percent. The reduction at the upper station is difficult to understand since drift samples indicated that this was the only station which did not show an increased drift of insects on spray days.

Reviewing Table 5 it is apparent that one of the check stations shows less increase in numbers of insects, between pre- and post-spray sampling periods, than do some of the test stations. Thus it would appear that malathion spray

had little effect on aquatic invertebrates. This phenomena points out the weakness of the method and substantiates the fact that bottom sample data alone should not be used as the sole basis for analysis. It also points to the need for pre-spray samples to be taken a year ahead of spraying.

Table 4. _____ Hours After Last Exposure _____ gray

Station	Sample Pre-gray	Sample gray	Sample Post-gray	Time of Sampling	Exposure										Total of Live Insects	Total of Dead Insects	Percent Alive	Percent Dead
					Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead				
Lower S. Fk.					5	1			2		6			1			.7	13.
Lower W.		X	X		7	6	2	184	1	2	3				13	1	.3	93.7
Lower N.		X	X			676				21		63		6		1756	0.0	100.0
Lower N. Fk		X		X	4	5		16		1	6	4			10	26	27.8	72.2
Lower S. Fk.		X	X		7	58		40		7	5	3			12	101	10.0	90.0
Lower S.		X		X	10	12	1	8	2		4		1	1	18	18	50.0	50.0
Lower Iron Cr. 1/		X	X		9	7			2	4	1			2	5	13	27.8	72.2
er Iron Cr. 2/		X	X			24			4			1	5	4	18	2	3 .3	61.7

1/ Coll ted July , 1

2/ Collected July 10, 1

Table 5. Numerical Counts of Aquatic Insects Collected from Bottom Samples

Station	July Pre-Spray Numbers						July Post-Spray Numbers						October Post-Spray Numbers						Total for July Pre-Spray	Total for October Post-Spray	Total for July Post-Spray
	Ephemeroptera	Plecoptera	Trichoptera	Diptera	Coleoptera	Other 1/	Ephemeroptera	Plecoptera	Trichoptera	Diptera	Coleoptera	Other 1/	Ephemeroptera	Plecoptera	Trichoptera	Diptera	Coleoptera	Other 1/			
Lower S. Fk.*	78	9	10	4	0	8	438	27	22	22	7	28	677	198	49	35	142	6	109	1107	544
Upper S. Fk.*	299	110	129	6	0	26	316	159	121	48	3	41	267	226	100	12	18	0	570	623	688
Total	377	119	139	10	0	34	754	186	143	70	10	69	944	424	149	47	160	6	679	1730	1232
Lower W. Fk.*	179	14	61	8	0	5	278	10	58	75	0	6	415	55	46	18	6	4	267	544	427
Upper W. Fk.*	177	125	98	8	0	14	200	95	151	12	0	17	103	107	66	1	0	2	422	279	475
Total	356	139	159	16	0	19	478	105	209	87	0	23	518	162	112	19	6	6	689	823	902
Lower N. Fk.*	168	37	74	2	6	19	263	15	102	25	13	50	693	225	260	20	5	4	306	1207	468
Upper N. Fk.*	44	12	11	4	0	11	138	29	25	7	2	56	362	71	61	12	14	4	82	524	257
Total	212	49	85	6	6	30	401	44	127	32	15	106	1055	296	321	32	19	8	388	1731	725
Lower Iron Cr.**	423	44	43	41	9	14	274	18	26	81	3	5	662	17	28	57	5	3	574	772	407
Cow Creek** (Control)	266	35	3	0	6	60	960	48	58	38	0	176	1904	358	93	39	17	7	370	2418	1280
Allison Cr.** (Control)	168	36	35	71	2	67	394	57	44	25	14	67	506	77	23	14	7	2	379	629	601

*Samples consist of 10 square feet of stream bottom per sampling period.
**Samples consist of 20 square feet of stream bottom per sampling period.
1/Primarily composed of round and flatworms.

• 6. Volumetric Measurements of Aquatic Insects Collected from [illegible] on Samples

Stations	July Pre-Spray Volumes						July Post-Spray Volumes						October Post-Spray Volumes						Total for July Pre-Spray	Total for July Post-Spray	Total for Oct. Post-Spray
	Ephemeroptera	Plecoptera	Trichoptera	Diptera	Coleoptera	Other 1/	Ephemeroptera	Plecoptera	Trichoptera	Diptera	Coleoptera	Other 1/	Ephemeroptera	Plecoptera	Trichoptera	Diptera	Coleoptera	Other 1/			
Lower South Fork*	1.0	0.1	T	T	[illegible]	T	3.3	0.2	1.9	0.1	T	T	2.9	0.5	T	0.4	0.2	T	1.1	5.5	4.0
Upper South Fork*	1.5	0.7	1.4	T	0	T	1.1	0.5	4.8	T	T	T	1.3	1.5	1.1	T	T	0	3.6	6.4	3.9
Total	2.5	0.8	1.4	T	0	T	4.4	0.7	6.7	0.1	T	T	4.2	2.0	1.1	0.4	0.2	T	4.7	11.9	7.9
Lower West Fork*	2.0	0.2	0.2	T	0	T	2.8	T	1.8	0.8	0	T	1.3	0.2	0.6	T	T	T	2.4	5.4	2.1
Upper West Fork*	2.0	0.6	0.8	T	0	T	0.6	0.1	1.8	T	0	T	0.2	0.1	0.1	T	0	T	3.6	2.5	0.4
Total	4.0	1.0	1.0	T	0	T	3.4	0.1	3.6	0.8	0	T	1.5	0.3	0.7	T	T	T	6.0	7.9	2.5
Lower North Fork*	4.1	1.3	2.2	T	0.1	T	2.1	1.0	5.5	T	T	T	2.9	0.7	4.2	T	T	T	7.7	8.6	7.8
Upper North Fork*	0.2	0.2	0.3	T	0	T	0.7	0.6	0.2	T	T	0.1	2.1	0.6	0.9	0.3	T	T	0.7	1.6	3.9
Total	4.3	1.5	2.5	T	0.1	T	2.8	1.6	5.7	T	T	0.1	5.0	1.3	5.1	0.3	T	T	8.4	10.2	11.7
Lower Iron Creek**	2.7	0.3	0.8	1.2	T	0.1	0.9	0.9	1.3	1.7	T	T	2.7	0.7	0.1	2.1	T	T	5.1	4.8	5.6
Cow Creek** (control)	5.4	T	0.1	0	T	0.3	0.2	T	0.5	0.2	0	T	7.5	0.8	1.5	3.2	T	T	5.8	10.9	13.0
Allison Creek** (control)	1.7	0.2	0.5	0.1	0.2	T	2.5	0.1	1.6	T	T	T	2.4	1.1	0.1	T	T	T	2.7	4.2	3.6

*Samples consist of 10 square feet of stream bottom per sampling period.
**Samples consist of 20 square feet of stream bottom per sampling period.
1/Primarily composed of round and flatworms.

V. LIVE-BOX TESTS

Methods

Live-box tests, using various sizes and species of fish, were conducted to test the toxicity of two low volume application rates of malathion spray. Live-boxes were placed within the different spray areas as well as two control streams (see maps). A total of three boxes, each with a different species of fish, were placed at six different sites. Check boxes were located in both Cow and Allison Creeks. Test fish were placed at the lower stations on each of the three spray areas, and on the lower end of Iron Creek proper.

Species of test fish consisted of rainbow trout four to seven inches long, chinook salmon fingerlings two to four inches in length, and steelhead fry approximately one inch long. Rainbow trout were furnished by the Idaho Fish and Game Department hatchery at Mackay, Idaho. Chinook fingerlings were taken from downstream migrant traps located on the Pahsimeroi, main Salmon and East Fork of the Salmon rivers. Steelhead fry were trapped in an artificial hatching channel located on the Lemhi river.

A total of 75 steelhead and rainbow trout, and 70 chinook salmon were placed in separate boxes at each of the six sites. Fish were placed in boxes five to six days before any spray operations commenced in order to eliminate sick or weak fish resulting from transportation and handling. All boxes were culled to 50 test fish, one to three days before spraying began. Boxes containing hatchery rainbow were culled to 54 fish. The four extra fish were later removed for brain samples to test cholinesterase levels.

Results. The results of live-box tests are shown in the following table.

Stream	Check or Test Location	Number of Fish Placed			Fish Left A/Culling B/Spraying			Fish Removed at End of Test Period					
								Rainbow		Chinook		Steelhead	
		Rainbow	Chinook	Steelhead	Rainbow	Chinook	Steelhead	Live	Dead	Live	Dead	Live	Dead
West Fork	Test	75	70	75	54*	50	50	49	1	50	0	49	1
North Fork	Test	75	70	75	54*	50	50	49	1	50	0	47	3
South Fork	Test	75	70	75	50	50	50	48	2	40	1	50	0
Lower Iron	Test	75	70	75	54*	50	50	48	2	50	0	50	0
Cow	Check	75	62	75	50	50	50	50	0	49	1	50	0
Allison	Check	75	70	75	50	50	50	49	1	50	0	49	1

*The extra four fish used for cholinesterase tests after being exposed.
Heavy losses of hatchery rainbow in the lower South Fork live-box.

Analysis of these data indicate that there were no adverse effects from malathion spray. The heavy losses of hatchery rainbow in the lower South Fork live-box before spraying was caused by fatigue. Once this box was given added protection from strong currents flowing through the box, mortality rates decreased.

A limited amount of physical and chemical water quality data were collected, figure 9. These data were collected to detect any significant changes which might influence the well being of test fish, and to determine if there were significant differences between the check and test streams.

Table 10. Water chemistry measurements in both test and control streams

Station	Max. Temp. (F°)	pH	Methyl orange alkalinity (p.p.m.)	Phenolphthalein alkalinity (p.p.m)	Dissolved oxygen (p.p.m.)
South Fork	56	7.5	68.4	0	11
West Fork	51	7.0	34.2	0	10
North Fork	52	7.0	27.4	0	10
Lower Iron Cr.	58	7.5	47.9	0	10
Cow Creek	53	7.0	47.2	0	9
Allison Creek	55	7.0	47.2	0	10

Biologist conducting water chemistry field tests

VI. TESTS FOR EFFECTS OF MALATHION ON THE CHOLINESTERASE LEVELS IN FISH BRAINS

Methods

Fish brain acetylcholinesterase is inhibited by organic phosphorous insecticides such a malathion. The degree of inhibition is a function of the concentration and chemical nature of the inhibiting substance as well as the extent of exposure. In addition, various species of fish react differently to the same insecticide with identical concentrations and exposures. Substantial reductions in brain enzyme activity will result in death. With lesser reductions in brain enzyme activity, full recovery may occur within a few days.

The purpose of the Iron Creek tests was to evaluate the effects of malathion on fishs' brains at 9 and 13 ounces per acre applications. Also to test differences, if any, between wild and hatchery reared fish of the same species.

Brain samples were taken from two sources. Brains were removed from hatchery reared rainbow trout which had been placed in live-boxes at the four different locations in the Iron Creek drainage. In addition, wild rainbow trout were captured by aportable electric fish shocker at the lower Iron Creek station.

The number of brain samples was limited due to laboratory capacity at Denver, Colorado. Therefore, pre-spray sampling was limited to three hatchery reared rainbow trout from each of four live-boxes. A total of four pre-spray samples was taken from wild rainbow trout from the lower end of Iron Creek. Replications of four fish from each live-box location and four wild fish from lower Iron Creek were sampled 24 to 168 hours after their last exposure to the insecticide.

Pre-spray brain samples were taken from fish that were killed and placed on ice. Specimens were then transported from the field to project headquarters where brains were removed approximately two hours later. Post-spray samples differed in that fish were transported alive to project headquarters, and brains were removed immediately after the fish were killed. All brain samples were placed in small vials with stoppers and placed under refrigeration at approximately 0° Fahrenheit. Samples were shipped by air freight to Denver, Colorado, where the Fish-Pesticide Research Laboratory of the Fish and Wildlife Service performed the chemical analyses.

Results

The following table shows the results of laboratory tests. The results are expressed in micromoles of a cetylcholine hydrolyzed in 30 minutes at 25°

centigrade per two milligrams of brain tissue. Under normal conditions fish brains which have not been affected by an organic phosphorus insecticide contain a greater amount of cholinesterase than do those brains which have been affected. Therefore, the amount of a cetylcholine hydrolyzed is normally greatest in the pre-spray bain samples. The table is confusing since it would appear that cholinesterase levels in brain samples from the West Fork and Lower Iron Creek stations were lower in pre-spray samples than in post-spray samples. This phenomena can be explained by the fact that it is quite normal to find a wide range of variation in the number of micromoles of a cetylcholine that are hydrolyzed in individual brain samples. Secondly, the number of samples per test lot is quite small. Lastly, the calculated insecticide concentrations, ranged from 0.4 ppm in the North Fork to 0.3 ppm in the West Fork. These concentrations are low enough and were flushed downstream fast enough that little or no effect would normally be expected.

Data for the North and South Fork stations do show a slight drop in cholinesterase levels. However, cholinesterase levels in both cases either approached or exceeded pre-spray levels within a 168 hour period after exposure.

Table 11 - Micromoles of acetylcholine hydrolyzed in rainbow fish brains from the Iron Creek drainage.

Station	Pre-spray Sample Averages	24-hour post-spray samples Averages	7-day post-Spray samples Averages
North Fork 1/	1.06	0.95	0.98
South Fork 1/	1.07	0.98	1.27
West Fork 1/	0.85	1.01	1.08
Lower Iron Creek 1/	0.79	0.98	1.13
Lower Iron Creek 2/	0.99	1.10	1.30

1/ Hatchery fish.

2/ Wild fish.

VII. CONCLUSIONS

1. Malathion spray killed considerable numbers of aquatic insects as evidenced by drift samples.

2. Some recovery can be expected among drifting aquatic insects when affected by malation spray.

3. Bottom fauna populations showed a general increase within three months after spray application.

4. No fish mortalities occurred within the test area which were attributable to malation spray.

5. Malathion spray had little or no effect on the cholinesterase levels of fish in the test area.

SUMMARY OF AQUATI INSECT DRI T SAMPLE DATA FOR ~~DAYTIME~~ nighttime SAMPLES (Each order of insects measured to the nearest tenth of a cubic centimeter per 5 minute drift sample)

Location	Sample Type	Sample Date 1/	No. of 5-min. drift Samples	Ephemeroptera Ave. No. per Sample	Ephemeroptera Ave. Vol. per Sample	Plecoptera Ave. No. per Sample	Plecoptera Ave. Vol. per Sample	Trichoptera Ave. No. per Sample	Trichoptera Ave. Vol. per Sample	Diptera Ave. No. per Sample	Diptera Ave. Vol. per Sample	Coleoptera Ave. No. per Sample	Coleoptera Ave. Vol. per Sample	Other Ave. No. per Sample	Other Ave. Vol. per Sample	Average Number of Insects per 5-min. Drift Sample	Average Number of Insects per 5-min. Drift Sample (Cubic Centimeters)
Lower North Fork	Pre-Spray	7/10/65	9	3.3	.01	0.4	T	0.2	T	1.1	T	0.1	T	-	-	5.2	0.1
	Spray Day	7/12/65	9	6.8	.07	10.2	.01	1.1	.01	4.2	T	-	-	-	-	22.3	.09
	Post Spray	7/19/65	9	1.2	.02	0.7	T	0.4	T	0.4	T	0.2	T	-	-	3.0	.02
Upper South Fork	Pre-Spray	7/11/65	9	8.9	T	1.3	T	0.9	T	0.1	T	-	-	-	-	11.2	T
	Spray Day	7/13/65	9	4.2	T	0.2	T	0.3	T	0.1	T	-	-	0.5	T	5.4	T
	Post Spray	7/14/65	9	5.6	T	0.4	T	0.2	T	-	-	-	-	0.2	T	6.4	T
Lower South Fork	Pre-Spray	7/11/65	9	7.4	.01	0.3	T	0.9	T	0.6	T	0.1	T	-	-	9.3	.01
	Spray Day	7/13/65	9	3.0	.01	1.0	T	0.3	T	0.1	T	0.1	T	-	-	4.6	.01
	Post Spray	7/14/65	9	2.6	T	0.2	T	0.4	T	-	-	0.1	T	0.1	T	3.3	T
Upper West Fork	Pre-Spray	7/7/65	9	2.6	.02	1.3	T	0.7	T	0.2	T	0.1	T	-	-	4.9	.02
	Spray Day	7/9/65	9	2.1	T	1.0	T	0.8	T	0.6	T	-	-	-	-	4.4	T
		7/10/65	9	2.6	T	1.2	T	0.6	T	0.3	T	-	-	0.3	T	5.0	T
		7/11/65	9	3.2	T	1.2	T	0.9	T	0.4	T	-	-	0.2	T	6.0	T
	Post Spray	7/12/65	9	1.5	T	0.8	T	0.3	.01	1.0	T	-	-	-	-	3.3	.1
Lower West Fork	Pre-Spray	7/7/65	9	13.6	.08	0.4	.01	1.6	.03	2.2	T	-	-	-	-	17.8	.12
	Spray	7/9/65	9	10.9	.08	0.8	T	1.1	T	3.3	.01	-	-	-	-	16.1	.09
	Day	7/10/65	9	13.4	.03	3.1	.02	4.7	.02	5.4	T	-	-	-	-	26.7	.08
		7/11/65	9	6.0	.04	0.7	T	1.2	T	2.9	.01	-	-	-	-	10.8	.06
	Post Spray	7/12/65	9	5.6	.07	0.4	T	0.6	T	2.7	T					9.2	.07
North Fork	Pre-Spray	7/10/65	9	15.1	T	1.6	T	0.6	T	2.9	T	-	-	-	-	20.1	T
	Spray Day	7/12/65	9	6.4	T	10.6	.02	1.3	T	5.1	T	-	-	-	-	23.4	.04
	Post Spray	7/13/65	9	2.0	.02	3.1	T	0.8	T	2.2	T	0.2	T	0.9	T	8.1	0.2

Summary of Aquatic Insect Drift Sample Data for Daytime Samples (Each order of insects measured to the nearest tenth of a cubic centimeter per 5 minute drift sample)

Location	Sample Type	Sample Date	No. of 5-min. Drift Samples	Ephemeroptera Ave. No. per Sample	Ephemeroptera Ave. Vol. Per Sample	Plecoptera Ave. No. Per Sample	Plecoptera Ave. Vol. Per Sample	Trichoptera Ave. No. Per Sample	Trichoptera Ave. Vol. Per Sample	Diptera Ave. No. Per Sample	Diptera Ave. Vol. Per Sample	Coleoptera Ave. No. Per Sample	Coleoptera Ave. Vol. Per Sample	Other Ave. No. Per Sample	Other Ave Vol. Per Sample	Average Number of Insects per 5-min. Drift Sample	Average Number of Insects per 5-min. Drift Sample (Cubic Cent
Upper West Fork	Pre-spray	7/7/65	10	5.6	T	5.5	T	0.5	T	0.4	T	0.2	T	-	-	12.2	T
		7/9/65	10	6.3	T	3.0	T	1.0	T	1.2	T	-	-	0.2	T	11.7	T
	Spray Day	7/10/65	10	5.8	T	3.3	T	0.7	T	1.1	T	0.1	T	-	-	11.0	T
		7/11/65	10	5.3	T	1.8	T	0.3	T	1.2	T	-	-	0.3	T	8.9	T
	Post Spray	7/12/65	10	3.3	.02	2.0	.02	0.2	T	0.6	T	-	-	-	-	6.1	.04
Lower West Fork	Pre-Spray	7/7/65	10	6.1	.01	0.6	T	1.3	T	2.5	T	-	-	-	-	10.5	.01
		7/9/65	10	8.2	.03	0.8	T	1.5	.01	1.4	T	-	-	-	-	11.9	.04
	Spray Day	7/10/65	10	31.1	.06	36.9	.04	7.0	.01	4.5	.01	0.1	T	-	-	79.6	.12
		7/11/65	10	7.5	T	1.1	T	2.3	.02	2.8	T	-	-	-	-	13.7	.02
	Post Spray	7/12/65	10	5.8	T	1.0	T	2.2	T	1.3	T	-	-	-	-	10.3	T
		7/23/65	7	5.0	.09	0.1	T	0.9	.01	0.3	T	-	-	0.1	T	6.4	.01
Upper North Fork	Spray Day	7/12/65	10	120.8	.22	63.5	0.1	8.6	.02	14.0	T	-	-	-	-	206.9	.34
Lower North Fork	Pre-Spray	7/10/65	10	2.5	T	.2	T	0.5	T	1.9	T	-	-	-	-	5.1	T
	Spray Day	7/12/65	10	95.1	.08	89.7	.25	5.9	T	10.8	.03	-	-	-	-	201.5	.36
	Post Spray	7/13/65	10	1.5	T	2.4	.01	0.4	T	2.0	T	-	-	-	-	6.3	.01
		7/23/65	8	4.8	.2	-	-	1.2	.06	0.2	T	-	-	-	-	6.2	.24
Upper South Fork	Pre-Spray	7/11/65	10	6.0	T	1.7	T	1.0	T	0.4	T	-	-	-	-	9.1	T
	Spray Day	7/13/65	10	60.8	.01	12.4	T	6.2	.02	1.3	T	0.1	T	-	-	70.8	.03
	Post Spray	7/14/65	10	5.3	T	1.6	T	1.3	T	0.4	T	-	-	-	-	8.6	T
Lower South Fork	Pre-Spray	7/11/65	10	5.9	T	0.3	T	0.3	T	0.8	T	0.1	T	-	-	7.4	T
	Spray Day	7/13/65	9	29.7	T	21.3	T	3.8	T	0.8	T	-	-	-	-	55.6	T
	Post Spray	7/14/65	10	1.4	T	0.4	T	0.1	T	0.2	T	0.1	T	-	-	2.2	T
		7/23/65	7	3.8	.09	0.1	T	0.3	T	-	-	0.3	T	0.3	T	4.9	.09
Lower Iron Creek	Pre-Spray	7/7/65	10	7.9	.02	0.7	T	1.6	.02	0.8	T	0.6	T	0.1	T	11.7	.04
	Spray Day	7/9/65	10	3.6	T	0.1	T	0.8	T	0.5	T	0.9	T	0.2	T	6.1	T
		7/10/65	12	27.2	.07	0.8	T	2.4	.03	0.8	T	1.8	.03	0.2	T	32.5	.13
		7/11/65	10	7.0	T	-	-	1.5	.08	1.4	T	1.1	T	0.2	T	11.2	.08
		7/12/65	11	119.1	.13	5.2	T	2.2	.04	1.5	T	1.0	T	0.4	T	129.4	.17
		7/13/65	10	2.0	T	0.7	T	0.7	T	0.4	T	0.6	T	0.4	T	4.8	T
	Post Spray	7/14/65	11	1.1	T	0.2	T	0.7	T	0.5	T	1.2	T	0.1	T	3.8	T
		7/23/65	9	0.6	T	-	-	-	-	0.2	T	-	-	-	-	0.8	T